Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 2431–2434

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis and SAR of Azalide 3,6-Ketal Aromatic Derivatives as Potent Gram-Positive and Gram-Negative Antibacterial Agents

Hengmiao Cheng,* John P. Dirlam, Carl B. Ziegler, Kristin M. Lundy, Shigeru F. Hayashi, Barbara J. Kamicker, Jason K. Dutra, Kirsten L. Daniel, Sheryl L. Santoro, David M. George, Camilla D. Bertsche, Subas M. Sakya and Melani Suarez-Contreras

Pfizer Global Research and Development, Groton Laboratories, Eastern Point Road, Groton, CT 06340, USA

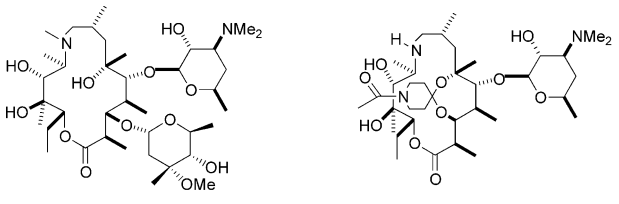
Received 14 February 2002; accepted 15 May 2002

Abstract—3,6-Ketals of 15-membered azalide pseudoaglycones are a novel series of macrolide antibiotics. The aromatic derivatives of the azalide 3,6-ketals demonstrated potent antibacterial activities against both Gram-positive and Gram-negative bacteria.

Mastitis is an inflammation of the mammary gland caused by a variety of bacterial pathogens. The most commonly isolated pathogens in dairy cows are *Staphylococcus aureus, Escherichia coli,* and several species of *Streptococcus*.[1] Mastitis has a significant economic impact on the world dairy industry due to costs associated with poor milk quality from infected cows, loss of milk from cows on treatment, and the cost of treatment. Between 30 and 50% of all dairy cows in the US are estimated to be affected by mastitis on a yearly basis.[1a,2]

Antibiotic therapy is commonly used to eliminate susceptible mastitis-causing pathogens from the udder. The most widely used antibacterial agents include penicillins, cephalosporins, pirlimycin, novobiocin, and dihydrostreptomycin. Intramammary infusion is widely used to deliver antibiotics into the infected udder. However, this treatment method may increase the chance of introducing environmental pathogens into the udder along with the drug. Moreover, none of the treatments are significantly effective against persistent *S. aureus* infections. Thus, an effective antibacterial agent administered as a single subcutaneous treatment at the beginning of the dry period would be preferred as a safe and convenient method of delivering an antimicrobial therapy.

Azalide antibiotics such as azithromycin **1** (Fig. 1) are well known for their broad-spectrum antibacterial activity and long tissue half-life.[3] In the search for novel azalide derivatives to treat bovine respiratory disease (BRD), Lundy et al. discovered that 3,6-ketal azalides are potent antibacterial agents against Gram-negative bacteria.[4] Subsequent cattle studies also demonstrated that 3,6-ketal azalides such as CP-456280 **2** (Fig. 1) are effective agents for treating BRD, which is caused typically by *Pasteurella multocida* and *Mannheimia* (*Pasteurella*) *haemolytica*.[5] In our search for a single-shot antibacterial agent using 3,6-ketal azalides as a template to treat dry cow mastitis, we discovered that introduction of an aromatic moiety to the piperidine nitrogen of the 3,6-ketal azalides increases their activity against *S. aureus*.



1 Azithromycin **2** CP-456280

Figure 1. 15-Membered azalide antibiotics.

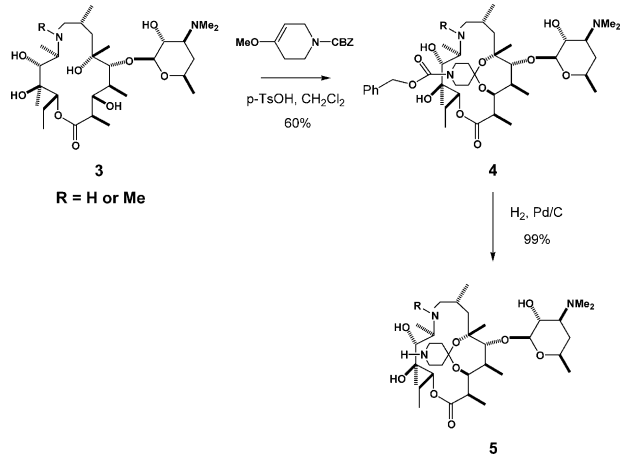
*Corresponding author. Fax: +1-860-441-6952; e-mail: henry_cheng@groton.pfizer.com

0960-894X/02/$ - see front matter
PII: S0960-894X(02)00434-1

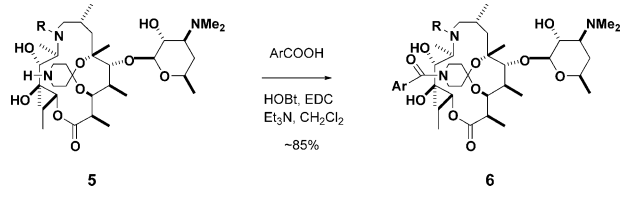
9a N–H and 9a N–Me 3,6-ketal templates were prepared as shown in Scheme 1.[6] 9a N–H or 9a N–Me descladinose azalide **3** was reacted with the methyl enol ether of *N*-CBZ piperidone in the presence of *p*-toluenesulfonic acid monohydrate to afford the 3,6-ketal derivative **4** in 60% yield. The CBZ protecting group was then removed by hydrogenolysis to generate the desired 3,6-ketal template in 99% yield.

Scheme 2 illustrates the general synthetic method for preparing piperidine-amide-3,6-ketal azalide derivatives. Under standard HOBt/EDC coupling conditions, the amide bond was formed only with piperidine nitrogen. The macrolide ring nitrogen was not affected when R′ is H at the 9a-position probably due to the steric hinderance from the macrolide ring.

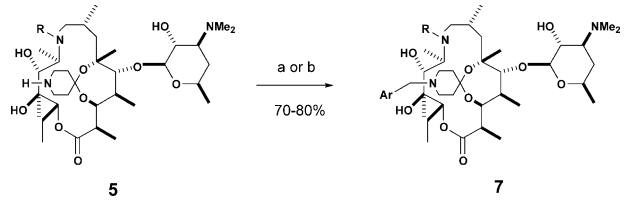
To make piperidine-amine-3,6-ketal azalide derivatives, two methods were employed as shown in Scheme 3. In method A, reductive amination was carried out using



Scheme 1. Synthesis of the N–H and N–Me azalide piperidine-3,6-ketal templates.



Scheme 2. General synthetic method for preparing piperidine-amide-3,6-ketal azalide derivatives.



Scheme 3. General synthetic method for preparing piperidine-amine-3,6-ketal derivatives. Reagents and reaction conditions: (a) ArCHO, $NaB(OAc)_3H$, AcOH, molecular sieves (3A), CH_2Cl_2; (b) $ArCH_2X$, Et_3N, CH_2Cl_2.

$NaB(OAc)_3H$ as the reducing agent. In method B, the piperidine nitrogen was alkylated following S_N2 reaction conditions with benzyl halides. Method A or B was chosen based on the availability of the aldehydes or the halides.

3,6-Ketal derivatives were tested against *P. multocida* (59A0067), *E. coli* (51A0150), and *S. aureus* (01A0785). Susceptibility testing was carried out by the microdilution method described in the NCCLS guidelines.[8] MIC (minimum inhibitory concentration) was determined at the lowest drug concentration that prohibits bacterial growth completely.

S. aureus intraperitoneal infection model. Twenty-gram female CF-1 mice were infected intraperitoneally (IP) with 1.6×10^5 CFU of *S. aureus* in 5% hog gastric mucin. Compounds were administered subcutaneously 0.5 h post-infection at doses of 10–40 mg/kg. The number of surviving mice was counted for 4 days, and the effective dose for 50% survival (ED_{50}) was calculated using a regression equation.

S. aureus intramammary infection model. Forty-gram female CD-1 lactating mice were infected in one mammary gland with ~38 CFU of *S. aureus* diluted in Dulbecco's phosphate buffered saline. Pups were removed at the time of infection. Compounds were given subcutaneously 0.5 h post-infection at doses of 5–30 mg/kg. The number of bacteria in the infected mammary gland was quantitated five days post-infection. Infected, non-medicated mice have 5×10^9 CFU/gland at the time of necropsy.

All the compounds in the piperidine-amide-3,6-ketal derivative series demonstrated antibacterial activity against Gram-positive and Gram-negative bacteria as shown in Table 1. Regardless of the size of the aromatic moiety, the ketal derivatives were very potent against *P. multocida* with MIC values ranging from 0.05 to 0.3 μg/mL. Compounds with an unsubstituted five-membered or six-membered aromatic moiety demonstrated good in vitro activity against *E. coli*. However, compounds with substituted or fused aromatic systems demonstrated decreased activity against *E. coli*. The MIC of the piperidine-amide-3,6-ketal derivatives against *S. aureus* varied from 0.2 to 3.13 μg/mL. There was no significant difference for in vitro activity between 9a N–H and 9a N–Me derivatives.

The in vivo activity of these ketal derivatives is also listed in Table 1. In general, more polar compounds were more potent in the *S. aureus* intraperitoneal infection model, and compounds with pyridine or quinoline moieties were the most active. The more polar 9a N–H derivatives were usually more active than 9a N–Me analogues. There is a trend that compounds with lower clogP values demonstrated better in vivo activity.

Our second in vivo model was a murine *S. aureus* intramammary infection (IMI) model that was designed to mimic bovine mastitis. In this model, potency is measured by decreasing bacterial count, as indicated by

Table 1. Biological data for piperidine-amide-3,6-ketals

Compd	Ar	R (9a)	In vitro MIC (μg/mL)			*S. aureus* IP model ED_{50} (mg/kg)	*S. aureus* IMI model Log_{10} CFU at 15 mg/kg	clogP
			P. multocida	*E. coli*	*S. aureus*			
1	N/A	Me	0.1	1.56	0.2	4.1	2.8	1.826
5	N/A	Me	0.05	0.2	6.25	—	—	1.454
8	Phenyl	H	0.1	1.56	0.78	20	2.9	1.847
9	Phenyl	Me	0.1	3.13	—	—	—	2.699
10	*m*-Methoxyphenyl	H	0.1	3.13	0.78	20	5.6	2.066
11	2-Pyridinyl	H	0.1	1.56	0.78	7.6	3.5	0.746
12	2-Pyridinyl	Me	0.1	1.56	1.56	12	2.9	0.746
13	3-Pyridinyl	H	0.1	3.13	1.56	6.4	3.2	0.746
14	3-Pyridinyl	Me	0.1	3.13	1.56	10	2.6	1.598
15	Pyrazine	H	0.1	1.56	1.56	6.1	1.3	−0.232
16	6-Hydroxy-2-pyridinyl	H	0.3	6.25	3.13	19	9.7	1.088
17	2-Chloro-3-pyridinyl	H	0.1	3.13	1.56	9.4	4.4	1.441
18	4,5-Dichloro-3-pyridinyl	H	0.1	3.13	3.13	>20	—	2.043
19	3-Furanyl	H	0.05	0.78	0.78	11	2.6	1.023
20	2-Furanyl	H	0.05	1.56	0.78	12	0.4	1.023
21	2-Furanyl	Me	0.05	0.78	—	>20	3.3	1.875
22	3-Methyl-2-furanyl	H	0.1	1.56	0.78	>20	—	1.522
23	2-Thienyl	Me	0.05	1.56	—	>20	—	2.476
24	2-Pyrrole	H	0.05	0.78	0.78	19	4.7	0.861
25	*N*-Methyl-2-pyrrole	H	0.1	1.56	0.78	19	2.4	1.102
26	2-Indole	H	0.1	3.13	0.78	>20	—	2.245
27	3-Indole	H	0.1	6.25	0.39	11	—	2.245
28	2-Quinoline	H	0.05	1.56	0.2	14	1.9	2.13
29	2-Quinoline	Me	0.1	6.25	0.39	>20	9.7	2.982
30	3-Quinoline	H	0.1	3.13	0.39	13	3.3	1.362
31	3-Quinoline	Me	0.1	6.25	0.39	>20	—	2.215
32	4-Quinoline	H	0.1	6.25	0.39	7.6	1.9	2.13
33	4-Quinoline	Me	0.1	6.25	0.39	20	2.5	2.982
34	Biphenyl-4-yl	H	0.2	6.25	0.78	—	—	3.735

Table 2. Biological data for piperidine-amine-3,6-ketals

Compd	Ar	R (9a)	In vitro MIC (μg/mL)			*S. aureus* IP model ED_{50} (mg/kg)	*S. aureus* IMI model Log_{10} CFU at 15 mg/kg	clogP
			P. multocida	*E. coli*	*S. aureus*			
1	N/A	Me	0.1	1.56	0.2	4.1	2.8	1.826
35	Benzyl	H	0.03	0.1	0.39	>20	—	3.436
36	Benzyl	Me	0.02	0.075	—	>20	7.3	4.288
37	*o*-Methoxybenzyl	H	0.03	0.1	0.39	—	—	3.355
38	*o*-Methoxybenzyl	Me	0.03	0.1	0.39	>20	—	4.207
39	*m*-Methoxybenzyl	H	0.03	0.3	0.39	—	—	3.355
40	*m*-Methoxybenzyl	Me	0.03	0.2	0.6	>20	—	3.471
41	*p*-Methoxybenzyl	H	0.01	0.2	0.2	—	—	3.355
42	*p*-Methoxybenzyl	Me	0.03	0.39	0.39	>20	9.7	4.207
43	*p*-Chlorobenzyl	H	0.01	0.2	—	—	—	4.149
44	*p*-Chlorobenzyl	Me	0.03	0.2	0.39	>20	9.7	5.001
45	3,4-Dichlorobenzyl	H	0.05	3.13	0.39	—	—	4.742
46	3,4-Dichlorobenzyl	Me	0.05	50	0.2	—	—	5.594
47	*p*-Cyanobenzyl	Me	0.03	0.39	0.78	22	—	3.721
48	4-Acetaminobenzyl	Me	0.05	0.39	—	9.6	9.7	3.307
49	3-Furanmethyl	Me	0.03	0.2	—	—	—	3.464
50	2-Pyridyl	H	0.03	0.2	0.6	<5	1.1	1.939
51	2-Pyridyl	Me	0.03	0.2	0.78	17	1.9	2.791
52	3-Pyridyl	H	0.03	0.78	0.39	<5	1.3	1.939
53	3-Pyridyl	Me	0.03	0.39	0.78	>20	—	2.791
54	4-Pyridyl	H	0.03	0.78	1.56	6.4	—	1.939
55	2-Quinolinemethyl	H	0.03	0.78	0.2	22	5.2	3.323
56	2-Quinolinemethyl	Me	0.03	0.78	0.39	>20	—	4.175
57	4-Quinolinemethyl	H	0.05	1.56	0.2	>20	—	3.323
58	4-Quinolinemethyl	Me	0.05	1.56	0.39	>20	—	4.175
59	Biphenyl-4-yl	Me	0.1	1.56	0.2	>20	—	6.176

a low $\log_{10}$ CFU value. A few compounds demonstrated very good efficacy. For example, when mice were treated with ketal derivative **20**, the observed $\log_{10}$ CFU was 0.4. This indicated that, among 10 mice treated with **20**, most of the murine mammary glands were free of *S. aureus* at day five.

Compounds in the piperidine-amine-3,6-ketal series have at least three basic nitrogens. The presence of a third basic nitrogen made these ketal derivatives more potent against both Gram-negative and Gram-positive bacteria as shown in Table 2. Most compounds demonstrated very potent activity against *P. multocida*. In general, the piperidine-amine-3,6-ketal derivatives were also very potent against *E. coli*. When the size of the aromatic moiety increased, the activity of the piperidine-amine-3,6-ketal against *E. coli* decreased. For example, the MIC for compound **36** against *E. coli* was 0.075 μg/mL. When the phenyl ring in compound **36** was substituted by another phenyl ring, the MIC of the resultant analogue **59** was increased to 1.56 μg/mL. Compared to the piperidine-amide derivatives, the piperidine-amine ketals demonstrated significantly more potent in vitro activity against *S. aureus*. This is consistent with the general observation that the more lipophilic the macrolide derivative is, the more potent its in vitro activity against *S. aureus*.[7] In general, the piperidine-amine derivatives have much higher clogP values than their amide analogues. For in vivo activity of macrolides, the general trend is that the more polar analogues usually demonstrate more potent activity.[7] This was also the case with this series of compounds. The piperidine-amine-3,6-ketals, which are more lipophilic than the piperidine-amide-3,6-ketal derivatives, were less active in the *S. aureus* intraperitoneal infection model. The exceptions are pyridyl derivatives in the 9a N–H series. For example, both compounds **50** and **52** demonstrated ED_{50}s less than 5 mg/kg. The same is true for their activity in the murine intramammary infection model; the $\log_{10}$ CFU for mice treated with **50** and **52** were 1.1 and 1.3, respectively.

In conclusion, 3,6-ketal azalides with potent in vitro activity against both Gram-negative and Gram-positive bacteria were discovered by derivatization of the piperidine ketal nitrogen with lipophilic aromatic moieties. There was no significant difference in the in vitro activity between 9a N–H and 9a N–Me analogues. However, compounds in the 9a N–H series usually demonstrated more potent activity in the in vivo models. Compounds **20**, **50**, and **52**, which have heterocyclic aromatic rings, demonstrated very good activity in the *S. aureus* intramammary infection model. They are promising candidates for further efficacy evaluations.

References and Notes

1. (a) Bramley, A. J.; Cullor, J. S.; Erskine, R. J.; Fox, L. K.; Harmon, R. J.; Hogan, J. S.; Nickerson, S. C.; Oliver, S. P.; Smith, L. K.; Sordillo, L. M. In *Current Concepts of Bovine Mastitis*, 4th ed.; National Mastitis Council, 1996; p 11. (b) Barkema, H. W.; Schukken, Y. H.; Lam, T. J. M.; Beiboer, M. L.; Wilmink, H.; Benedictus, G.; Brand, A. *J. Dairy Sci.* **1998,** *81,* 411.
2. (a) Hogan, J. S.; Smith, K. L.; Hoblet, K. H.; Schoenberger, P. S.; Todhunter, D. A.; Hueston, W. D.; Pritchard, D. E.; Bowman, G. L.; Heider, L. E.; Brockett, B. L.; Conrad, H. R. *J. Dairy Sci.* **1989**, *72*, 1547. (b) Clements, M. In *Animal Pharm Reports, Bovine Mastitis: Products and Markets*; PJB: 1998, p 9.
3. Bright, G. M.; Nagel, A. A.; Bordner, J.; Desai, K. A.; Dibrino, J. N.; Nowakowski, J.; Vincent, Lawrence; Watrous, R. M.; Sciavolino, F. C.; English, A. R.; Retsema, J. A.; Anderson, M. R.; Brennan, L. A.; Borovoy, R. J.; Cimochowski, C. R.; Faiella, J. A.; Girard, A. E.; Girard, D.; Herbert, C.; Manousos, M.; Mason, R. *J. Antibiot.* **1988**, *41*, 1029.
4. (a) Minich, M. L.; Lundy, K. M. *Abstract of Papers*, Structural Elucidation of Descladinose Azithromycin-3,6-(Azacyclohexyl) Ketal by One- and Two-D NMR Spectroscopy, 218th National Meeting of the American Chemical Society, New Orleans, LA, Aug. 22–26, 1999. (b) Minich, M. L.; Lundy, K. M.; Rafka, R. J.; Morton, B. J. *Abstract of Papers*, Azalide 3,6-Ketals: Discovery of a Novel Class of Azalide Antibiotics, 218th National Meeting of the American Chemical Society, New Orleans, LA, Aug 22–26, 1999.
5. Lundy, K. M.; Minich, M.; Jaynes, B.; Hayashi, S.; Kamicker, B.; Bertsche, C.; Cheng, H.; George, D.; Morton, B.; Pratt, B.; Rafka, R.; Santoro, S.; Silvia, A. *Abstract of Papers*, Azalide 3,6-Ketals: Synthesis and SAR of a Novel Class of Macrolide Antibiotics, 2000 Pacifichem Meeting, Honolulu, Hawaii, December 14–19, 2000; Poster number 264.
6. Cheng, H.; Bertinato, P. A.; Bertsche, C. D.; Daniel, K.; Dutra, J. K.; George, D.; Hayashi, S. F.; Kamicker, B. J.; Lundy, K. M.; Minich, M. L.; Morton, B. J.; Pratt, B.; Rafka, R. J.; Sakya, S.; Santoro, S. L.; Suarez-Contreras, M.; Vamvakides, N.; Ziegler, C. B. Azalide 3,6-Ketals: SAR in *An Aromatic Analogue Series with Potent Gram-positive and Gram-negative Activity*, 2000 Pacifichem meeting, Honolulu, HI, Dec 14–19, 2000; Posternumber 265.
7. McFarland, J. W.; Berger, C. M.; Froshauer, S. A.; Hayashi, A. F.; Hecker, S. J.; Jaynes, B. H.; Jefson, M. R.; Kamicker, B. J.; Lipinski, C. A.; Lundy, K. M.; Reese, C. P.; Vu, C. B. *J. Med. Chem.* **1997**, *40*, 1340.
8. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals; approved standard, M31-A, Vol. 19, No. 11, June, 1999.